PERTURBATION APPROACH TO RESONANCE SHIFT OF WHISPERING GALLERY MODES IN A DIELECTRIC MICROSPHERE AS A PROBE OF A SURROUNDING MEDIUM

5   ## § 0.1   RELATED APPLICATIONS

This application claims benefit to U.S. Provisional Application Serial No. 60/440,236, titled "Perturbation Approach to Resonance Shift of Whispering Gallery Modes in a Dielectric Microsphere as a Probe of a Surrounding Medium," filed on January 15, 2003, and listing

10   Stephan Arnold, Iwao Teraoka and Frank Vollmer as inventors (referred to as "the '236 provisional"). That application is incorporated herein by reference. The scope of the present invention is not limited to any requirements of the specific embodiments described in that application.

15   ## § 0.2   FEDERAL FUNDING

This invention was made with Government support and the Government may have certain rights in the invention as provided for by grant number BES0119273 by the National Science Foundation.

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§ 1.   BACKGROUND OF THE INVENTION

§ 1.1   FIELD OF THE INVENTION

25   The present invention concerns analysis of chemicals and/or biological materials such as, for example, measurement, detection, etc. In particular, the present invention concerns instrumental analysis of chemicals and/or biological materials.

§ 1.2   BACKGROUND INFORMATION

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There are many applications in which chemicals or biological substances need to be analyzed. A wide assortment of instrumentation is available to the analyst. In some cases, the instrument is used to characterize a chemical reaction between the analyte and an added reagent;

in others, it is used to measure a property of the analyte. Instrumental analysis is subdivided into categories on the basis of the type of instrumentation employed. Such categories of instrumental analysis include spectral methods, electroanalysis methods, and separatory methods. Each of these methods has limits, such as costs, size, portability, time, sensitivity, etc.

5   Separatory instruments may use chromatography or mass spectrometry for example. High performance liquid chromatography (HPLC) is a popular method of analysis, and is often used to analyze drugs, foods, beverages, environmental samples, etc. (See, e.g., U.S. Patent Nos. 3,985,021 and 5,795,469 (both incorporated herein by reference.) Unfortunately, detectors used in liquid chromatography are often bulky.

10   In view of the foregoing, better methods of instrumental analysis and the associated instrumentation are needed. In the case of liquid chromatography, less bulky, yet accurate, detectors would be desirable.

§ 2. SUMMARY OF THE INVENTION

15   The present invention provides new methods of instrumental analysis that determine a refractive index, or a refractive index profile, of an analyte. The present invention may make such determinations using a shift in a property of light, such as a wavelength or resonance frequency shift of light in a microsphere, where the microsphere is immersed in the analyte, as in the case HPLC for example.

20   The present invention also provides examples of instrumentation used to practice such new methods, such as in the context of HPLC for example.

§ 3. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates sensitivity factors f_{TE} (solid lines) and f_{TM} (dashed lines) of resonance

25   frequency of TE and TM modes, respectively, in a microsphere to a uniform refractive index change in the surrounding medium, plotted as a function of size parameter $k_0 a$. The lines are for the first three orders ($n = 1$, 2, and 3) for $m_1 = 1.47$ and $m_2 = 1.33$.

Figure 2 illustrates an expected sensing limit $\Delta m_{2,min}$ of refractive index change in the surrounding medium in microspheres of different $k_0 a$ for the first three modes. Solid lines are

30   from the intrinsic linewidth of resonance. Dashed lines denote the sensing limit for current DFB laser driver's resolution, $\Delta k / k_0 = 10^{-8}$.

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Figure 3 is a diagram illustrating an exemplary refractive index detector consistent with the present invention.

Figure 4 is a flow diagram of an exemplary method that may be used to detect a refractive index of an analyte in a manner consistent with the present invention.

5   Figure 5 illustrates a refractive index profile across a microsphere–surrounding medium interface.

Figure 6 illustrates frequency shift due to refractive index change of $\Delta m_2(r) = \Delta m_2(\infty)[1 - \exp(-\Gamma(r-a))]$, reduced by the shift due to a constant Δm_2, as a function of $1/\Gamma$. All the lines share $a = 100$ μm and $m_2 = 1.33$. The values of the other parameters are indicated in the legend.

10   Figure 7 is a diagram illustrating an exemplary refractive index profile detector consistent with the present invention.

Figure 8 is a flow diagram of an exemplary method that may be used to determine a refractive index profile of an analyte in a manner consistent with the present invention.

15   § 4. DETAILED DESCRIPTION

The following description of embodiments consistent with the principles of the invention provides illustration and description, but is not intended to be exhaustive or to limit the invention to the precise form disclosed. Modifications and variations are possible in light of the following teachings or may be acquired from practice of the invention. For example, although a series of

20   acts may have been described with reference to a flow diagram, the order of acts may differ in other implementations when the performance of one act is not dependent on the completion of another act. Further, non-dependent acts may be performed in parallel.

No element, act or instruction used in the description should be construed as critical or essential to the invention unless explicitly described as such. Also, as used herein, the article "a"

25   is intended to include one or more items. Where only one item is intended, the term "one" or similar language is used.

The present invention may determine a refractive index, or a refractive index profile, of an analyte using a shift in a property of light, such as a wavelength or resonance frequency shift of light in a microsphere, where the microsphere is immersed in the analyte, as in the case HPLC

30   for example. The resonance frequency is sensitive to the environment in close vicinity of the sphere surface. Any change in the vicinity can be detected as a frequency shift. The changes will include adsorption of molecules onto the sphere surface and the concentration change in the

surrounding solution. A perturbation approach may be used to evaluate the resonance frequency shift of a WGM. In such an approach, the frequency shift $\delta\omega$ is associated with the perturbation in the energy, δE, of a single-photon resonant state due to adsorption of a dielectric nanoparticle by

$$\hbar\delta\omega = \delta E = -\tfrac{1}{2}\mathrm{Re}[\delta\mathbf{p}\cdot\mathbf{E}^*], \tag{1}$$

5

where $\delta\mathbf{p}$ is the induced dipole moment in the nanoparticle, and $\mathbf{E}$ is local field within the original mode. The formulation was applied to a microsphere with a mono-layer coverage by protein molecules and a sphere with a single protein molecule adsorbed onto the equator. In the past, perturbation approach was applied to resonance modes in a spherical microcavity with

10 reflective interior surface. (See, e.g., the articles, P. J. Wyatt, "Scattering of electromagnetic plane waves from inhomogeneous spherically symmetric objects", Phys. Rev., Vol. **127**, pp. 1837-1843 (1962), and D. Q. Chowdhury, S. C. Hill. P. W. Barber, "Morphology-dependent resonances in radially inhomogeneous spheres", J. Opt. Soc. Am. A, Vol. **8**, pp. 1702-1705 (1991). Formulas were obtained for the frequency shift due to a spatial change in the refractive

15 index within the microcavity. These aforementioned formulations, however, do not allow one to evaluate the frequency shift due to changes in the medium surrounding the microsphere.

The use of a microspheres to detect a substance is described in U.S. Patent Application Serial No. 10/096,333 (incorporated herein by reference), titled "DETECTING AND/OR MEASURING A SUBSTANCE BASED ON A RESONANCE SHIFT OF PHOTONS

20 ORBITING A MICROSPHERE," filed on February 12, 2002, and listing Stephen Arnold and Iwao Teraoka as inventors, U.S. Patent Application Serial No. 10/690,979 (incorporated herein by reference), titled "ENHANCING THE SENSITIVITY OF A MICROSPHERE SENSOR," filed on October 22, 2003 and listing Stephen Arnold, Iwao Teraoka and Frank Vollmer as inventors, U.S. Patent Application Serial No. 10/735,247 (incorporated herein by reference),

25 titled "USING A CHANGE IN ONE OR MORE PROPERTIES OF LIGHT IN ONE OR MORE MICROSPHERES FOR SENSING CHEMICALS USHC AS EXPLOSIVES AND POISON GASES," filed on December 12, 2003 and listing Stephen Arnold, Iwao Teraoka, Yoshiyuki Okamoto and Frank Vollmer as inventors, and U.S. Provisional Application Serial No. 60/443,736 (incorporated herein by reference), titled "PERTURBATION OF OPTICAL

30 CAVITIES BY DNA HYBRIDIZATION," filed on January 30, 2003 and listing Stephen Arnold, Iwao Teraoka and Frank Vollmer as inventors. As will be apparent to one skilled in the

art, some of the methods and apparatus, or portions thereof, described in the foregoing applications may be used in concert with the present invention.

The perturbation exploited by various embodiments of the present invention is a small change in the refractive index. The change can be either within the sphere or in the surrounding medium. When applied to a uniform change in the refractive index of the surrounding medium, the formula gives the same result as the one obtained from the resonance formula for a homogeneous surrounding medium. Some embodiments of the present invention use the frequency shift, since it can be measured accurately (the resolution is better than 1/5 of the linewidth) and is relatively unaffected by auxiliary effects which are important in linewidth measurements, such as scattering and absorption by inhomogeneity of the sphere and the surrounding medium.

General formulas for the frequency shift for TE and TM modes derived in the '236 provisional are applied to (1) determine a uniform refractive index change in the medium surrounding one or more microspheres (caused by, for instance, a change in the surrounding fluid) in § 4.1 below, and (2) to determine a refractive index profile near the surface of one or more micropheres in § 4.2 below. Other parameters (such as a and m_1) are assumed not to change, unless otherwise mentioned. In the following, "l" may be used to denote an angular momentum quantum number; "r" may be used to denote a radial distance from the center of a microsphere, "a" may be used to denote the radius of a microsphere, "m_1" may be used to denote the refractive index of a microsphere, "m_2" may be used to denote the refractive index of medium surrounding a microsphere, and art-recognized symbols may be used to represent other quantities or attributes.

§ 4.1   UNIFORM CHANGE IN REFRACTIVE INDEX OF THE SURROUNDING MEDIUM

§ 4.1.1 DERIVATION

In the following vacuum wavevector k is defined as $k = 2\pi/\lambda$, where λ is the wavelength. Therefore, the relative frequency shift is related to the relative wavelength shift as $\delta k/k_0 = -\delta$ λ/λ_0, where λ_0 is the wavelength at resonance before shift, and $\delta\lambda$ is the wavelength shift. $\delta\lambda$ may be measured by monitoring the intensity of light transmitting the fiber that is coupled to the

microsphere (radius a, refractive index m_1) as the wavelength is scanned in a narrow range. The resonance wavelength is continuously followed.

For a uniform change Δm_2 in the exterior refractive index, the shift can be estimated using the resonance condition. The comparison of the latter result with the one to be obtained from the general formulas of the first-order perturbation validates the general formulas. In Eq. 11 of the '236 provisional that gives the resonance condition for the TE mode, change k_0 to $k_0 + \Delta k$ and change m_2 to $m_2 + \Delta m_2$. Collecting the first-order terms, the fractional shift $\Delta k/k_0$ is calculated as:

$$
\left(\frac{\Delta k}{k_0}\right)_{TE} = -\frac{m_2 \Delta m_2}{m_1^2 - m_2^2}\left[\frac{l(l+1)}{(m_2 k_0 a)^2} - 1 + \frac{1}{m_2 k_0 a}\frac{\chi_l'}{\chi_l} - \left(\frac{\chi_l'}{\chi_l}\right)^2\right]
$$

$$
= -\frac{m_2 \Delta m_2}{m_1^2 - m_2^2}\left[\frac{\chi_{l+1}\chi_{l-1}}{\chi_l^2} - 1\right], \qquad (2)
$$

where Eq. C6 of the '236 provisional was used, and $\chi_{l-1}(z)$, $\chi_l(z)$, and $\chi_{l+1}(z)$, defined by Eq. 12 of the '236 provisional, are evaluated at $z = m_2 k_0 a$. For the TM mode, use of Eq. 15 of the '236 provisional leads to:

$$
\left(\frac{\Delta k}{k_0}\right)_{TM} = -\frac{m_2 \Delta m_2}{m_1^2 - m_2^2}\frac{\dfrac{l(l+1)}{(m_2 k_0 a)^2} - 1 - \dfrac{1}{m_2 k_0 a}\dfrac{\chi_l'}{\chi_l} - \left(\dfrac{\chi_l'}{\chi_l}\right)^2}{\dfrac{l(l+1)}{(m_1 k_0 a)^2} + \left(\dfrac{\chi_l'}{\chi_l}\right)^2}. \qquad (3)
$$

Now the general formulas in the first-order perturbation are used to evaluate the fractional frequency shift. For the TE mode,

$$
\langle k_0|m\delta m|k_0\rangle = m_2 \Delta m_2 [B_l(k_0)]^2 \int_a^\infty [\chi_l(m_2 k_0 r)]^2 dr
$$

$$
= [B_l(k_0)]^2 \frac{a}{2}m_2 \Delta m_2\left[-\chi_l'^2 + \left(\frac{l(l+1)}{(m_2 k_0 a)^2} - 1\right)\chi_l^2 + \frac{\chi_l \chi_l'}{m_2 k_0 a}\right], \qquad (4)
$$

where the same trick used in evaluating Eq. A9 of the '236 provisional was used to force the integral to converge. The term was neglected due to the upper limit of the integral. With Eq. 26 of the '236 provisional, the same equation as Eq. 2 is obtained.

For the TM mode, the formula given by Eq. 40 of the '236 provisional is used, because 5 $\delta m(r)$ remains finite at large r. The integrals are evaluated as follows:

$$\left\langle k_0 \left| m^{-1} \delta m \right| k_0 \right\rangle = [A_l(k_0)]^2 \frac{a}{2} \frac{\Delta m_2}{m_2} \left[-\chi_l'^2 + \left(\frac{l(l+1)}{(m_2 k_0 a)^2} - 1 \right) \chi_l^2 + \frac{\chi_l \chi_l'}{m_2 k_0 a} \right], \tag{5}$$

$$\left\langle k_0 \left| \frac{1}{k_0^2 m^2} \frac{d(\delta m / m)}{dr} \frac{d}{dr} \right| k_0 \right\rangle = \frac{\Delta m_2}{k_0^2 m_2} \int_0^\infty \frac{1}{m^2} T_0 \frac{dT_0}{dr} \delta(r-a) dr = \frac{\Delta m_2}{k_0 m_2^2} [A_l(k_0)]^2 \chi_l \chi_l'. \tag{6}$$

With Eqs. 27 and 40 of the '236 provisional, the same expression as Eq. 3 is obtained.

When $k_0 a \gg 1$, use of Eqs. B5 and B7 of the '236 provisional simplifies Eqs. 2 and 3 to:

$$\left(\frac{\Delta k}{k_0} \right)_{TE} \cong -\frac{m_2 \Delta m_2}{m_1^2 - m_2^2} \left[(l + \tfrac{1}{2})^2 - (m_2 k_0 a)^2 \right]^{-1/2}, \tag{7}$$

$$\left(\frac{\Delta k}{k_0} \right)_{TM} \cong -\frac{m_2 \Delta m_2}{m_1^2 - m_2^2} \frac{2 \left(l + \tfrac{1}{2} \right)^2 \big/ (m_2 k_0 a)^2 - 1}{\left[\left(l + \tfrac{1}{2} \right)^2 - (m_2 k_0 a)^2 \right]^{1/2} \left[\frac{\left(l + \tfrac{1}{2} \right)^2}{(m_1 k_0 a)^2} + \frac{\left(l + \tfrac{1}{2} \right)^2}{(m_2 k_0 a)^2} - 1 \right]}. \tag{8}$$

The shift occurs in the same direction to the two modes. For the first-order mode, $l \cong m_1 k_0 a$ and these expressions are further simplified to:

$$\left(\frac{\Delta k}{k_0} \right)_{TE} \cong -\frac{m_2 \Delta m_2}{(m_1^2 - m_2^2)^{3/2}} \frac{1}{k_0 a}, \tag{9}$$

and

$$\left(\frac{\Delta k}{k_0} \right)_{TM} \cong -\frac{m_2 \Delta m_2}{(m_1^2 - m_2^2)^{3/2}} \left(2 - \frac{m_2^2}{m_1^2} \right) \frac{1}{k_0 a}. \tag{10}$$

Thus, the ratio of the TM shift to the TE shift at $k_0 a \gg 1$ is greater than unity for the first-order mode.

Figure 1 shows a plot of sensitivity factors $f_{TE} \equiv -(\Delta k/k_0)_{TE}/[m_2 \Delta m_2 (m_1{}^2 - m_2{}^2)^{-1}]$ and

$f_{TM} \equiv -(\Delta k/k_0)_{TM}/[m_2 \Delta m_2 (m_1{}^2 - m_2{}^2)^{-1}]$ for the first, second, and third-order modes ($n = 1, 2,$

and 3, respectively) as a function of size parameter $k_0 a$. Equations 2 and 3 with $m_1 = 1.47$

(silica) and $m_2 = 1.33$ (water) were used for the calculation. At $k_0 a \gg 1$, both f_{TE} and f_{TM}

5   decrease as $\sim(k_0 a)^{-1}$, in agreement with the asymptotic expressions (Eqs. 9 and 10). In this

range, $f_{TE} < f_{TM}$ for each mode. The higher-order mode (greater n) experiences a greater shift

compared at the same $k_0 a$, but the difference quickly disappears with an increasing $k_0 a$. Both

f_{TE} and f_{TM} peak at around $k_0 a \cong 76$ for the first-order mode. This is due to a term neglected in

Eqs. 7 and 8. The peak moves left and up, extending the nearly straight section, when m_1 is

10   increased, thus more strongly confining the resonant photon by a greater contrast of refractive

index.

For a given sphere with $a/\lambda \gg 1$, $\Delta k/k_0$ is proportional to the wavelength λ. The shorter

the wavelength, the weaker is the effect by the surroundings. This fact is related to the

penetration depth of the evanescent field from a high refractive index medium to a low refractive

15   index medium when total internal reflection occurs.

Toward the low end of $k_0 a$ in the plot, the sensitivity factor is high, but the resonance

peak may be too broad for any meaningful detection of Δm_2 by measuring $\Delta k/k_0$. The detection

limit may be arbitrarily set to the width w of the peak (in terms of $k_0 a$) which is given by:

$$w = \frac{2m_2}{m_1{}^2 - m_2{}^2} \frac{1}{[\chi_l(m_2 k_0 a)]^2},$$ (11)

20   for the TE mode. Then, the smallest $\Delta m_{2,\min}$ that can be detected is estimated as:

$$\Delta m_{2,\min} = 2 / [k_0 a \chi_l{}^2 f_{TE}] = 2 / [k_0 a (\chi_{l+1}\chi_{l-1} - \chi_l{}^2)].$$ (12)

Likewise, for the TM mode,

$$\Delta m_{2,\min} = \frac{2}{k_0 a \chi_l^2 f_{TM}} \frac{1}{\dfrac{\left(l+\frac{1}{2}\right)^2}{(m_1 k_0 a)^2} + \left(\dfrac{\chi_l'}{\chi_l}\right)^2} \cdot \tag{13}$$

Solid lines in Figure 2 show $\Delta m_{2,\min}$ for the first three orders of the TE mode. The first-order mode is the most sensitive compared at the same $k_0 a$. At $k_0 a = 400$, $\Delta m_{2,\min}$ as small as 2×10^{-8} can be detected. Note that $\Delta m_{2,\min}$ decreases rapidly with an increasing $k_0 a$, which

5   may be due to smaller leakage of the photon energy.

There may be another limit on $\Delta m_{2,\min}$ due to the laser linewidth or the source wavelength fluctuations, whichever is greater. In the study of protein adsorption, a DFB laser ($\lambda = 1.34 \mu$m) was used. (See, e.g., the paper, F. Vollmer, D. Braun, A. Libchaber, M. Khoshsima, I. Teraoka, S. Arnold, "Protein detection by optical shift of a resonant microcavity", Appl. Phys.

10   Lett., Vol. **80**, pp. 4049-4057 (2002).) The source wavelength fluctuations may surpass the laser linewidth. The fluctuations were about 10^{-5} nm, which translates into $\Delta k/k_0 \sim 10^{-8}$. Dashed lines in Figure 2 represent $\Delta m_{2,\min}$ imposed by this restriction. They are nearly flat between $\sim10^{-6}$ and $\sim10^{-7}$ in the range of the plot. It is now apparent that the second limit may restrict the detection limit. Accordingly, it may be advisable to decrease the source fluctuations.

15

§ 4.1.2 EXEMPLARY APPARATUS

Figure 3 is an exemplary apparatus that may be used to detect a change in the refractive index in the medium surrounding microsphere 350. The micosphere 350 is optically coupled

20   with fiber 340. A laser 330, such as a variable wavelength laser diode for example, may be used to inject light into the fiber 340. A detector 360, such as a photodiode for example, may be used to detect properties of light in the fiber 340. As shown, the microsphere 350 may be provided in fluid passage 310 through which fluid (eluent) 320 flows.

This apparatus may be used to detect a change in the refractive index in the medium

25   surrounding the microsphere 350. Equations 9 and 10 above indicate that the relative shift, $\Delta k/k_0$ is proportional to Δm_2. A typical situation might be a detector used in liquid chromatography to detect a refractive index (RI detector). The microsphere 350 may be placed in a fluid constantly

washed by the eluent. Usually, a constant mobile phase is flowing around the microsphere 350. As eluent 320 containing an analyte reaches the microsphere 350, the resonance starts to shift. When the surrounding fluid returns to the mobile phase, the shift returns to zero. The height of the shift is proportional to the concentration of the analyte in the eluent. This example assumes a

5   spatially uniform refractive index.

For RI sensing, the surface of the microsphere 350 should be modified to avoid adsorption of a chemical. For instance, reaction of the silanols on the surface of silica (or hydroxyls on the surface of other oxides) with octyldimethylchlorosilane changes the surface from silanol to octyl (usually referred to as C8 in HPLC). In organic solvent, such a surface will

10   repel most of the analytes.

A computing device (not shown), such as a personal computer for example, may function to (a) control the light source 330, (b) record shifts of some property of light, (c) determine a (change in) refractive index, and/or (d) identify an analyte using the determined (change in) refractive index.

15

§ 4.1.3 EXEMPLARY METHOD

Figure 4 is a flow diagram of an exemplary method 400 that may be used to detect a refractive index of an analyte in a manner consistent with the present invention. A light source (e.g., 330) is applied (Block 405) and light is detected (e.g., with detector 360) (Block 410). One

20   or more resonance frequencies are recorded. (Block 415) The sensing head (e.g., 350) is then surrounded with a liquid of interest (e.g., eluent containing analyte 320). (Block 420) A light source is again (or continuously) applied (Block 425) and light is again (or continues to be) detected (Block 430). One or more resonance frequencies are again (or continuously) recorded. (Block 435) The change in resonance frequency (or change in wavelength) is then determined.

25   (Block 440) A change in refractive index (e.g., of the eluent to the analyte) may then be determined using the change in resonant frequency. (Block 445)

§ 4.2   DEPLETION OF REFRACTIVE INDEX AT THE INTERFACE

A change in the profile of the refractive index in the medium surrounding the

30   microsphere may be generated. Figures 5 and 6 illustrate the profile. RI is plotted as a function of the distance from the microsphere surface. Since how $\Delta k/k_0$ depends on the profile is

different for different wavelengths of light, mode orders (n), and refractive indices of the microsphere, this dependence can be used to determine the profile.

§ 4.2.1 DERIVATION

5   Using different values of k_0a, especially λ, enables WGM to be a sensor for the refractive index profile of the surrounding medium near the surface. When the exterior medium is a solution of macromolecules or a suspension of particles, the refractive index of the solution may change as a function of the distance from the sphere surface. If the solute has a positive differential refractive index and does not interact with the microsphere except steric hindrance,

10   the refractive index may have a profile as depicted in Figure 5. Although this mean-field refractive index profile may not be valid for suspension of large solid particles with a large refractive index difference, it will nonetheless be a good approximation for solutions of macromolecules and suspension of swollen gel particles and vesicles.

It is convenient to separate $\Delta m_2(r)$ into two parts: $\Delta m_2(r) = \Delta m_2(\infty) - [\Delta m_2(\infty) - \Delta m_2(r)]$. In

15   the first-order perturbation, each term gives an independent frequency shift. Thus,

$$\frac{\Delta k}{k_0} = \left(\frac{\Delta k}{k_0}\right)^{\text{inf}} - \left(\frac{\Delta k}{k_0}\right)^{\text{dep}}, \tag{14}$$

where the first term is given by Eqs. 2 and 3 ($\Delta m_2(\infty)$ in place of Δm_2) for the TE and TM modes, respectively. The second term decreases rapidly to zero at long distances. For the TE mode,

20   $$\left(\frac{\Delta k}{k_0}\right)^{\text{dep}}_{\text{TE}} = -\frac{\langle k_0 | m_2[\Delta m_2(\infty) - \Delta m_2(r)] | k_0 \rangle}{\langle k_0 | m_2^2 | k_0 \rangle} = -\frac{m_2 \int_a^\infty [\chi_l(m_2 k_0 r)]^2 [\Delta m_2(\infty) - \Delta m_2(r)] dr}{(a/2)[\chi_l(m_2 k_0 a)]^2 (m_1^2 - m_2^2)}. \tag{15}$$

For the TM resonance, $(\Delta k / k_0)^{\text{dep}}_{\text{TM}}$ can be calculated similarly by using Eq. 41 of the '236 provisional.

Numerical calculation was done for a profile $\Delta m_2(r)$ that changes as:

$$\Delta m_2(r) / \Delta m_2(\infty) = 1 - \exp[-\Gamma(r - a)], \tag{16}$$

11

for $r > a$. The lines in Figure 6 are plots of Δk of the first-order TE mode, reduced by its $(\Delta k)^{\text{inf}}$,

for $a = 100$ μm and $m_2 = 1.33$ but various values of m_1, λ, and n. Comparing the five solid lines,

note that a shorter wavelength or a microsphere with a greater refractive index is less sensitive to

a deeper depression. The higher-order mode can explore a deeper depression. The values of

5   parameters used in the figure are currently accessible. Use of microsphere sensors of different λ

and m_1 and analysis of various orders of resonance will allow the depth of depression and its

profile to be estimated. In a solution of macromolecules, for instance, the molecular weight

distribution may be determined just by dipping the sensor heads into the solution. A more

elaborate expression for $\Delta m_2(r)$ may be necessary for that purpose.

10

§ 4.2.2 EXEMPLARY APPARATUS

Figure 7 illustrates an exemplary measurement system that allows a scan of the resonance

spectrum having resonances coming from different mode orders to be performed. Analysis of all

of the information obtained may be used to determine the refractive index profile. One or more

15   microspheres 750 are optically coupled with one or more fibers 740. One more lasers 730, each

having a different wavelength may be used to inject light into the fibers 740 via fiber coupler and

splitter 760. One or more detectors 760, such as photodiodes for example, may be used to detect

properties of light in the fibers 740. As shown, the microspheres 750 may be provided in fluid

passage 710 through which fluid (eluent) 720 flows. Note that when different components (e.g.,

20   lasers, microspheres, etc.) are used, certain operations can occur in parallel. Alternatively, or in

addition, operations can be performed in series. For example, a first laser wavelength may be

injected into a first fiber coupled with a first microsphere and a first sensor while a second (and

perhaps further) laser wavelength may be injected into a second (and perhaps further) fiber

coupled with a second (and perhaps further) microsphere and a second (and perhaps further)

25   sensor. Alternatively, or in addition, a first laser wavelength may be injected into a first fiber

coupled with a first microsphere and a first sensor at a first time, and a different laser wavelength

may be injected into the first fiber coupled with the first microsphere and the first sensor at a

later time, and so on.

The center wavelength of the light sources 730 may be, for example, blue, red, 980nm, 1350nm, 1550nm, etc. The different microspheres 750 may be of different materials, such as silica, polystyrene, sapphire, etc.

A computing device (not shown), such as a personal computer for example, may function to (a) control the light sources 730, (b) record shifts of some property of light, (c) determine a refractive index profile, and/or (d) identify an analyte using the determined refractive index profile.

§ 4.2.3 EXEMPLARY METHOD

Figure 8 is a flow diagram of an exemplary method 800 that may be used to determine a refractive index profile of an analyte in a manner consistent with the present invention. As indicated by loop 805-835 a number of acts are performed for each of a plurality of wavelengths. (Recall, e.g., laser sources 730.) The light source at the wavelength is applied to each of a plurality of microspheres (e.g., 750). (Block 810) As indicated by loop 815-830 for each of the plurality of microspheres, light is detected (e.g., with 760) (Block 820), and one or more resonant frequencies are recorded (Block 825). The sensing head(s) (e.g., 750) are then surrounded with a liquid of interest (e.g., eluent containing analyte 720). (Block 840)

As indicated by loop 845-875 a number of acts are performed for each of a plurality of wavelengths. The light source at the wavelength is applied (or continues to be applied) to each of a plurality of microspheres. (Block 850) As indicated by loop 855-870, for each of the plurality of microspheres, light is (or continues to be) detected (Block 860), and one or more resonant frequencies are recorded (Block 865). Changes in the resonant frequencies (or changes in the wavelength are determined. (Block 880) A refractive index profile is then determined using the changes in the resonant frequencies for various wavelengths, for various microspheres, and/or for various {wavelength,microsphere} pairs. (Block 885) This determination may be made using the results of test for many polymer standards of a narrow molecular weight distribution. Figure 6 shows the expected $\Delta\lambda/\lambda$ for a profile shown in Figure 5. The

determination of a refractive index profile using changes in resonant frequencies is a reverse operation of this.

If the method of Figure 8 is practiced with the apparatus of Figure 7, five light sources and three microspheres may be used for example. The microspheres are made of transparent materials of different refractive indices, for instance, silica, polystyrene, and sapphire.

§ 5.   CONCLUSIONS

The embodiment of the present invention discussed in § 4.1 above can be used to construct tiny refractive index detectors that may replace bulky detector widely used in liquid chromatography. The embodiment of the present invention discussed in § 4.2 allows small instruments to measure size distribution of suspensions and molecular weight distribution of polymers. The measurement may be instantaneous. A polymer with a higher molecular weight may have a depression in the refractive index over a longer distance compared with a polymer with a lower molecular weight. A polymer with a different molecular weight distribution has a different refractive index profile near the surface.